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(54) **Glyphosate tolerant 5-enolpyruvyl-3-phosphoshikimate synthase.**

(57) Glyphosate-tolerant 5-enolpyruvyl-3-phosphoshikimate (EPSP) synthases, DNA encoding glyphosate-tolerant EPSP synthases, plant genes encoding the glyphosate-tolerant enzymes, plant transformation vectors containing the genes, transformed plant cells and differentiated transformed plants containing the plant genes are disclosed. The glyphosate-tolerant EPSP synthases are prepared by substituting an alanine residue for a glycine residue in a conserved sequence found between positions 80 and 120 in the mature wild-type EPSP synthase.

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## EUROPEAN SEARCH REPORT

Application Number

EP 88 87 0096

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
X	EP-A-0 218 571 (MONSANTO CO.) * Example 8 *	5,6,8,9 ,11,13, 16,24, 26,34, 40,41, 44-47	C 12 N 15/00 C 12 N 9/10 C 12 N 5/00 A 01 H 1/00
Y		15,17- 23,25, 27-33, 35-39, 42,43	
O,X	--- FEDERATION PROCEEDINGS, vol. 45, no. 6, 1986, page 1506, abstract no. 148, PROCEEDINGS OF THE 76TH ANNUAL MEETING OF THE AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, Washington, DC, 8th - 12th June 1986; G.M. KISHORE et al.: "Isolation purification and characterization of a glyphosate tolerant mutant Escherichia-coli 5 enolpyruvylshikimate-3-phosphate synthase" * Abstract *	5,6	
A	--- NATURE, vol. 317, no. 6039, October 1985, pages 741-744, London, GB; L. COMAI et al.: "Expression in plants of a mutant aroA gene from Salmonella typhimurium confers tolerance to glyphosate" * Whole document *	9-47	
A	--- EP-A-0 115 673 (CALGENE) * Whole document * --- -/-	1-8,44- 47	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 21-09-1990	Examiner MADDOX A.D.
<b>CATEGORY OF CITED DOCUMENTS</b>			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... & : member of the same patent family, corresponding document	



DOCUMENTS CONSIDERED TO BE RELEVANT															
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)												
P,X	THE EMBO JOURNAL, vol. 7, no. 5, 1988, pages 1299-1305, IRL Press ltd, Oxford, GB; G. DELLA-CIOPPA et al.: "Import of a precursor protein into chloroplasts is inhibited by the herbicide glyphosate" * Page 1300, right-hand column - page 1301, left-hand column *	1,2,5,7,8													
P,A	CHEMICAL ABSTRACTS, vol. 109, 1988, page 192, abstract no. 105880j, Columbus, Ohio, US; & JP-A-63 98 388 (KIRIN BREWERY CO., LTD) 28-04-1988 * Abstract *	1,3,5,6,7,44-47													
O,X	PROC. BR. CROP. PROT. CONF.-WEEDS, 1987, vol. 2, pages 463-471; R. FRALEY et al.: "Genetically-engineered herbicide tolerance - technical and commercial considerations"	1,3,5,6,8-11,13,14,16,24,26,32,34,40-													
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int. Cl.4)												
Place of search THE HAGUE		Date of completion of the search 21-09-1990	Examiner MADDOX A.D.												
<table border="0"><tr><td>CATEGORY OF CITED DOCUMENTS</td><td>T : theory or principle underlying the invention</td></tr><tr><td>X : particularly relevant if taken alone</td><td>E : earlier patent document, but published on, or after the filing date</td></tr><tr><td>Y : particularly relevant if combined with another document of the same category</td><td>D : document cited in the application</td></tr><tr><td>A : technological background</td><td>L : document cited for other reasons</td></tr><tr><td>O : non-written disclosure</td><td>&amp; : member of the same patent family, corresponding document</td></tr><tr><td>P : intermediate document</td><td></td></tr></table>				CATEGORY OF CITED DOCUMENTS	T : theory or principle underlying the invention	X : particularly relevant if taken alone	E : earlier patent document, but published on, or after the filing date	Y : particularly relevant if combined with another document of the same category	D : document cited in the application	A : technological background	L : document cited for other reasons	O : non-written disclosure	& : member of the same patent family, corresponding document	P : intermediate document	
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-1- (WPAT)  
 AN - 88-339892/48  
 XR - 92-339628 94-159181  
 XRAM- C88-150184  
 XRPX- N88-257722  
 TI - Mutant 5-enol:pyruvyl-3-phospho:shikimate synthase enzymes - used to obtain glyphosate tolerant plants while maintaining synthase catalytic activity  
 DC - D16 P13  
 PA - (MONS ) MONSANTO CO  
 IN - KISHORE GM, SHAH DM  
 NP - 10  
 NC - 19  
 PN - EP-293358-A 88.11.30 (8848) 41p E  
 AU8816601-A 88.12.01 (8904) E  
 JP01039984-A 89.02.10 (8912)  
 DK8802856-A 89.01.05 (8915)  
 ZA8803735-A 89.04.26 (8922) E  
 CN1032030-A 89.03.29 (9014)  
 US4971908-A 90.11.20 (9049) E  
 EP-293358-B1 94.07.06 (9426) 50p E C12N-015/52  
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 ES2058338-T3 94.11.01 (9444) C12N-015/52  
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 CT - 5.Jnl.Ref A3...9050 EP-115673 EP-218571 J63098388 No-SR.Pub 02Jnl.Ref  
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 FD - DE3850527 Based on EP-293358; ES2058338 Based on EP-293358  
 IC - A01H-001/00 A01H-005/00 C07G-017/00 C07H-021/04 C12N-005/00 C12N-009/10  
 C12N-015/52  
 AB - (EP-293358-A)  
 A method for producing glyphosate-tolerant 5-enolpymivyl-3-phosphoshikimate (EPSP) synthase enzymes comprises substituting an alanine residue for the second glycine residue in the amino acid sequency -L-G-N-A-G-T-A- located between positions 80 and 120 in a mature wild type EPSP synthase sequence.  
 Also claimed is a glyphosate-tolerant EPSP synthase enzyme which contains the amino acid sequence -L-G-N-A-A-T-A- between positions 80 and 120 in the mature EPSP synthase sequence, a plant gene encoding the enzyme and a DNA sequence encoding the enzyme.  
 USE/ADVANTAGE - Glyphosate tolerant plants can be obtd. which produce mutant EPSP synthase enzymes which exhibit a lower affinity for glyphosate while maintaining catalytic activity. Suitable plants are e.g. soybean, cotton, flax, tomato, potato, tobacco, wheat and rice.  
 (Dwg.0/12)

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